THE CHICAGO MEDICAL SCHOOL
QUARTERLY
VOLUME 21 JANUARY, 1961 NUMBER 2

METHODS AND CONCEPTS USED IN THE STUDY OF SHOCK*

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Many of our past concepts relating to shock and shock-like states were the outgrowth of observations of a clinical nature upon wounded, injured or hemorrhaged patients. Important as these observations have been, there has also grown up as weeds about the good seed, an appalling amount of confusion and contradiction. The diversity of opinion regarding the treatment of shock during and after the first World War emphasized the lack of uniformity or standardization in the methods for the investigation of this disorder. About this time a committee on shock was organized by the National Research Council to coordinate laboratory studies[1]. Attempts to develop standardized methods were renewed so that some common meeting ground for purposes of communication and comparison might be framed.

One of the oldest methods for production of experimental shock consisted of exposure and manipulation of the intestines of an animal[2]. A second method produced shock by trauma from tourniquets placed about the lower extermities[3]. Various other methods for the production of shock included trauma with a hammer or blunt instrument[4], aseptic burns produced with diathermy[5], pressure on the hind leg by winding stretched rubber tubing[6], or by means of a vise[7], long bone fracture[8], and scalding[9].

Most of these preparations have been largely superceded by the Wiggers or Western Reserve method[10] which consisted of hemorrhaging animals to a given arterial blood pressure for a period of time with limits established to produce a uniformly fatal preparation or a desired percentage of survivals. In small animals a fairly uniform tumbling injury or "drum shock" has been produced using the Noble-Collip "drum" in which rats of a given strain were tumbled for a certain specified number of times over a given period of time. It was after large scale and considered efforts that the latter two preparations were developed and adopted in most laboratories studying shock.

The Western Reserve preparation or variations of it, has been used for dogs and other large animals as the basic test situation. Blood samples were obtained to measure various plasma constituents and on the basis of elevation or depression of these plasma components metabolic or hemodynamic defects were inferred. Another approach using

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This work was supported in part by grants from the United States Public Health Service, numbers A-3115 and H-4809. The assistance of the Upjohn Company, the Eli Lilly Company, and the Charles H. and Rachael M. Schwab Memorial Foundation is gratefully acknowledged.

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this hemorrhage preparation employed comparison of the mean arterial pressure and the pressure in various vascular efflux systems draining individual organs; e.g., portal vein pressure, inferior vena cava pressure, central venous or right atrial pressure[12,13]. Cardiac output and total peripheral resistance were measured[10] and in some instances, blood flow through specific organs such as heart[14], liver[15] and kidney[16] have been estimated.

Using strict aseptic techniques, germ free rats were subjected to shock[17] by the Western Reserve method[10] and by the Noble-Collip drum[11]; the appearance of bacteria and bacterial products (endotoxins) were observed and correlated with survival rates[18]. Furthermore, antibiotics[19], properdin levels[20], autonomic blocking agents[21], chlorpromazine[22], immunization against various bacteria, etc., have been used in an attempt to improve the number of survivals. Within this basic preparation portal caval shunts[23], perfusion of the liver[24] or intestines as well as many other experimental or remedial measures have been examined.

Numerous attempts have also been undertaken to pinpoint certain biochemical lesions which may predispose or produce the lesion of irreversible shock. Among these investigations are the measurement of oxygen consumption[25], as well as bile flow, certain specific hepatic function tests such as clearance of bilirubin, amino acids[26], and bromsulphalein[27] as well as the synthesis of fibrinogen[28], prothrombin[29], and urea[26].

Following sacrifice of the animal in the irreversible hemorrhagic-shocked state, liver samples have been taken in order to measure various intracellular constituents. Depression in the activity of the enzymes, cocarboxylase and cozymase[30], the depletion of glycogen and certain high energy chemical stores such as creatine phosphate, adenosine triphosphate, and adenosine diphosphate[31] as well as an increased concentration of creatinine, inorganic phosphate and nitrogenous metabolities[32] have been observed in the tissues. Another approach to a biochemical lesion has been entertained by Engel[33] who used a metabolic blocking agent, fluoroacetate and showed that by blocking the Krebs cycle a state of shock could be induced.

All of these methods or approaches have, in their historical past, added considerably to our knowledge and understanding of the shock syndrome. But underlying the multipicity of studies, there are relatively few well-defined preparations or approaches to the problem. Most of these studies have been done using, as a yardstick, the survival rate (or length of survival time) after hemorrhagic shock; from comparison of the control series with a series given an experimental variable, information relating to the causes, development, or treatment of shock are inferred.

A second more direct approach involves measurements of the physiologic reactions or responses of the animal or measurement of the circulating plasma constituents. Another "direct approach" attempts to measure tissue concentrations and **in vitro** reactions rates of various substances. Interpretations of results by the latter method are open to the criticism that **in vitro** reactions do not approach normal physiological equilibria conditions. Furthermore, the concentration of substances taken at autopsy may not reflect the dynamic interrelationships of the living state, and, moreover, these observed changes may be the result rather than the cause of shock.

The results of approaches using the **in vivo** animal in which the circulating plasma is measured may also be variously interpreted. The difficulties in interpretation bear a striking similarity to that great controversy which raged for many decades regarding diabetes, i.e., whether the elevation in blood sugar was the result of overproduction or underutilization of glucose. The measurement of plasma concentration alone sheds very little light on this type of question, since the circulating plasma concentration itself represents a balance between production and utilization.

The elevation of free amino acids, blood urea nitrogen, glucose, lactic acid, pyruvic acid and the fall of fibrinogen, prothrombin and plasma pH suggest a metabolic defect but do not establish

where, that is, in what organ or organ system, this defect may be, nor whether it is a defect of production or utilization. Moreover, this approach does not answer the still more pressing problem of supplying a quantitative answer to the rate of production and the rate of utilization of a given plasma constituent. One can not assume that all cells in the body act similarly, that all cells, tissues, or organs produce, utilize, clear or destroy a given plasma constituent at the same rate as other cells, tissues or organs.

A Proposed Approach for Investigation of Shock

Amid the multiplicity of experiments using these two basic preparations or their numerous modifications and variations, there appears to be a paucity of fresh and different approaches. The fundamental problems of shock still remain unanswered: what are the factors which go to produce the irreversible stage in hemorrhagic shock?, what are the specific factors which are primary or causative and which are secondary or contributory?, and to what extent quantitatively do each of these contribute to the development of this syndrome? We wish to propose a concept of regional hemodynamics and metabolism as an appropriate approach toward the solution of these critical problems.

The characteristics of the demise from hemorrhage have been extensively studied and observed; the results of numerous investigations undertaken in different disciplines with differing points of view indicate that the liver plays a predominant role in the development of irreversible shock[34]. Fine[35] has called attention to the liver as the primary locus for factors leading to irreversibility; Wiggers points to the increased portal vein pressure as one of the most critical measurements of impending demise. Furthermore, he and others have suggested or inferred that the hepatic resistance is somehow increased in normovolemic shock. Presumably, an increased hepatic resistance may be the cause or, at least, the harbinger of circulatory failure.

However, actual measurement of hepatic resistance in the normal, unanesthetized dog as compared with the dog in a shocked state, await the development of more precise regional flow measurements. As Wiggers states, "Most of our information regarding the great Splanchnic Circuit still remains based on circumstantial rather than on quite certain experimental evidence."[10]

In order to answer some of these questions we[36,37,38] have devised methods and approaches that will allow precise measurement of the hemodynamic or physiologic parameters of the major organs or organ systems, and at the same time we have attempted to quantify metabolic reactions of these organs. Because the liver is the central organ of metabolism and because it has been incriminated as the primary locus of the factors leading to irreversibility, it was regarded as the logical place to start. Both the hemodynamic and metabolic defects in this organ are compared as well as concomitant metabolic and hemodynamic events occurring in other organ systems or in the remainder of the body.

Using this approach these metabolic and hemodynamic events are measured in a third type of reference, namely, as a function of time — so that the sequence of events may be derived. From this sequence of events information may be obtained as to which events are primary or causative and which events are secondary or compensatory. Moreover, in this broad approach where quantitative regional measurements are desired, it is entirely possible that one may determine when a compensatory reaction with survival value becomes sufficiently magnified that it becomes an overcompensatory reaction jeopardizing survival.

It must be realized that shock (a poor term) may be considered a "waste basket" syndrome where many different causes can conceivably produce the same irreversible outcome. The specific aim of our present studies are to elucidate the events and their mechanisms, irrespective of whether they may be initiated by blood loss, bacterial action, endotoxins, metabolic poisons, histamine, peptone, or other substances which may give rise to an anaphylactic-like state. Even though

the initiating causes may be legion, it is hoped that there may be some physiological and biochemical events in common and that elucidation of the dynamic interrelationships governing these events may be of value to the understanding and treatment of this common surgical problem.

Our distant and illusive goal, yet to be fully achieved, is to characterize and quantify each of the hemodynamic and metabolic events leading to the irreversible stage of shock and then to establish the precise physiological and biochemical mechanisms involved in the development of these events. Perhaps then, more rational therapeutic measures may be entertained.

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A TENTATIVE VIEW OF THE CAUSES AND DEVELOPMENT OF IRREVERSIBLE SHOCK*

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Justification for the measurement of regional hemodynamics and metabolism has been presented as an approach toward elucidation of the factors involved in the development of irreversible shock[1]. In the pursuit of this concept, methods and techniques were devised which included a method of catheterizing the major hepatic vessels in the dogs for use in the chronic unanesthetized state[2]; modified and improved methods for measurement of hepatic blood flow[3] and a method for the perfusion of the liver **in vivo**[4].

Initial studies on the regional metabolism and hemodynamics of the dog subjected to hemorrhagic shock have been presented[5] and compared with the response to neurohumeral[6] and other factors known to be operative in hemorrhagic shock. The hepatic microcirculation of the dog, rat and mouse during the development of hemorrhagic shock has been examined[7] and the effects of the viscosity changes through the addition of high molecular dextran were observed[8].

In this communication, a tentative hypothesis is presented which is consistent with the observed phenomena and which has served as our **ad hoc** or working hypothesis during these studies. Conclusions on this, or indeed, any rapidly developing research area are necessarily tentative; like a cathedral which is always "a building" yet never completed, research efforts are subject to continuous revision, modifications as well as major alterations. With the humility borne of numerous experiences wherein tentative hypotheses have been modified during the course of investigations to such an extent that they bear little resemblance to the initial views, the following broad picture is represented as our present view of the factors and mechanisms responsible for the development of irreversible shock.

With the onset of blood loss, initial compensatory changes occur; among these are the prompt increase in the output of catacholamines by the adrenal gland[9]. With an increase in catacholamine output there occurs a redistribution of cardiac output producing both a higher percentage of cardiac output traversing the liver as well as a greater absolute rate of hepatic blood flow[6]. With further blood loss, however, this increased hepatic blood flow due to cardiac output redistribution is no longer seen. There are at least two reasons for this; first and quite obviously there is less blood volume to redistribute and, secondly, with this early compensatory catachol effect there arises an increase in the hepatic venous resistance. After continued blood loss, the hepatic blood flow gradually and progressively falls concomitantly with the increase in resistance to flow across the hepatic vascular bed.

As these flow changes take place the metabolic effects of epinephrine on the hepatic tissues itself are increasingly

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felt. Initially, the liver may put out potassium and glucose although the circulating plasma potassium output of the liver could only be picked up by minute to minute observations. The influence on the circulating plasma potassium and glucose is quite small because of the larger reservoir of intercellular potassium throughout the body with which equilibrates the circulating plasma potassium. Similarly, small increases in circulating plasma glucose are quickly taken up by the peripheral tissues. As the terminal stage is approached with increased and increasing catachol levels the potassium and glucose output by the liver is increasingly apparent.

At this juncture it is of aid to our understanding of the problem to review the effects of epinephrine, per se, on hepatic hemodynamics and metabolism. A small injection of epinephrine produces an increased hepatic blood flow, an increased potassium output and an increased glucose output from the liver; the first two of these effects are maximum approximately 60 seconds after injection. With the constant infusion of epinephrine in approximately the same dose one finds a slightly different sequence, the potassium output by the liver appears to be confined to the first minute of the constant infusion, similarly, the hepatic blood flow is found predominantly in the initial phase of the infusion although depending on the dose administered, an increased hepatic blood may be observed throughout the course of the infusion.

Several explanations of these phenomena are possible. With the action of the catachol in producing an hepatic potassium output, certain reactions to this phenomena occur such that rapid reequilibration of the increased plasma potassium occurs with intracellular potassium both in the liver and throughout body tissues. The second explanation arises from the fact that with increased epinephrine administration there occurs also an increased capacity of the organism to destroy it. An explanation of this latter phenomena is found in classic description of enzyme-substrate kinetics in which the enzyme is substrate dependent. Increased substrate (in this case, epinephrine) concentration, therefore, produces an increased activity of the enzyme and an increased rate of its destruction. Since monamineoxidase is present in great abundance in most tissues as are the enzymes which destroy epinephrine by orthomenthylation, the rate limiting factor is largely that of the concentration of the epinephrine. Thus, with increasing concentrations of the epinephrine there is an increasing capacity of the body to destroy it. These phenomena, in general, apply to a fairly wide range of concentrations.

When a constant infusion of epinephrine is administered there is an initial potassium response. If a second injection of epinephrine is given on top of this continuous infusion, a second similarly short lived potassium response is seen. Thus, with increasing or accelerating rates of epinephrine output (at the same time blood volume is shrinking due to the hemorrhage itself) the increasing concentrations of epinephrine will rather markedly affect both hepatic potassium and glucose output. This occurs with increasing intensity toward the terminal stage of shock and persists to the final agonal event. With prolonged hemorrhage there appears to be no exhaustion of the capacity of the adrenal gland for continued production of catachols.

With increased hepatic blood flow there is concomitant increase in hepatic venous resistance. Presumably the mechanisms of these two events is the same epinephrine response. The increase in portal vein pressure and hepatic venous resistance is the weak link in the chain leading to irreversible shock. Much of the increased hepatic blood flow is shared by the abdominal viscera as well as the liver since the portal vein blood flow represents blood which has already gone through the nonhepatic splanchnics and which will eventually traverse the liver. Thus, the increased blood flow through the gut may in and of itself contribute to the portal venous pressure rise. This, together with the increased hepatic venous resistance leads to the development of the well known phenomenon of se-

questration of blood in the splanchnic area. Splanchnic sequestration of blood has been frequently stated to occur with shock, but the events which lead to its formation and the mechanisms or dynamics which contribute to its development are ill defined and poorly studied.

In order to examine these events in more detail, studies of the microcirculation were undertaken with the hope that some of the principal factors involved in a development of microcirculatory events could be correlated with measurements of hemodynamic events simultaneously occurring in the whole organ.

With single injection of small doses of epinephrine an initial increase in hepatic blood flow through the sinusoids and hepatic venous collecting system is readily observed microscopically; although it is difficult to quantify in precise units or terms. With increasing doses of epinephrine, microcirculatory events take place in the following order. First, there is a slight dilitation in the hepatic sinusoids occurring with the increase in hepatic blood flow. Following this dilatation there appears to be a congestion; as the dilatation gives way to congestion, sinusoidal blood flow decreases. The congestion first observed in the sinusoids, is later seen in the small collecting venules of the hepatic efflux system.

As this reaction becomes more intense, a definite clumping of red cells or aggregation, is observed in the sinusoids; later, clumps of red cells are seen interspersed with clear areas of plasma. At first these clumps move more slowly than the normal blood flow but as the lesion progresses there is more aggregation, less movement (i.e., flow) and further progressive involvement of the hepatic venous efflux system. Observation of the sinusoids during this stage for periods up to a half hour or more revealed no movement of the red cell masses in the stagnant, congested sinusoids. Other sinusoids nearby may be sluggish but still have movement of red cells, or red cell masses.

There are occasions when very high doses of epinephrine rapidly administered produce blanching of the sinusoidal areas following an initial stage of decrease flow and red cell aggregation. This phenomenon is consistent with the hypothesis that epinephrine in low doses produces constriction of the outlet sinusoidal sphincters, but after large doses there is a generalized constriction of the hepatic microcirculation including the portal vein system and the sinusoids themselves. While this latter situation may occur with high doses rapidly administered, it is probably an exception to the general rule that with exogenous administration of physiologic doses of epinephrine over a prolonged period or the prolonged increased endogenous production of epinephrine such as are observed in clinical states of shock result in red cell aggregation or sludging, decreased blood flow in the sinusoids and hepatic congestion.

As one observes shock by hemorrhage over a period of time the identical sequence of events in the hepatic microcirculation is observed. This again suggests that the untoward events leading to the irreversible stage of shock may largely be the effects of prolonged epinephrine stimulation. In summary, the animal dies in hemorrhagic shock the circulation does not come to an abrupt halt like the end of the "wonderful one-horse shay," nor does it slowly coast to a halt as a car running out of gas. It stops with a definite pattern or sequence. In order to understand this pattern one must, yet again, delve into another basic science area in order to identify and evaluate the factors involved in the development of these microcirculatory phenomena.

Flow of blood or any fluid through a rigid system of tubes has been described by Poiseuille. Within certain limits the flow of blood through blood vessels in biological systems is also governed by these laws. Poiseuille described the interrelationship between velocity, viscosity and the diameter of the tube; with decreasing velocity of blood flow there is an increased viscosity and this effect is related to the fourth power of the diameter of the vessel. Changes in viscosity related to alterations in flow have a direct relationship with the capacity of

the plasma to keep red cells in suspension. This red cell suspension capacity is by definition inversely related to the sedimentation rate of the blood; sludging or aggregation of the red cells results from the inability of a moving column of blood to keep the red cell suspended in plasma. In a wider blood vessel such as the sinusoid, there is already a decrease in velocity, and when blood flow or cardiac output diminishes below a certain critical level, then the slower flow rate (velocity) produces an even greater viscosity which results in aggregation of red cells. This is illustrated by the experiment where highly viscous, high molecular weight dextran was added to increase viscosity with the resultant decrease in flow and aggregation of red cells; this defect was corrected by the administration of a low viscosity, low molecular weight dextran.

It is most significant that these changes were found to take place in the hepatic sinusoids. They have previously been described in the peripheral circulation such as the sclera in burns. The post capillary venules comprised that element of the vascular tree which has the greatest cross sectional area of the circulatory tree. They have, therefore, the widest vessel diameter relative to the flow rate. Important as these peripheral vascular changes are, even more important are the changes occurring in the central or vital organs.

Those factors which are operative to produce the lesions in the post capillary venules are even more operative with respect to the hepatic sinusoids since it is in the sinusoids that the rate of flow relative to the diameter of the vessel is the most critical of any area in the body. It is, therefore, quite easy to understand the reasons why this lesion should first occur in the liver, and quite possibly the spleen or other sinusoidal organs. When this lesion occurs, moreover, the increased portal venous pressure is further intensified.

This additional increase in portal vein pressure produces a physiological block or dam effect resulting in the backing up of blood in the splanchnic area drained by the portal vein. Thus, stagnation or a decreased flow in this area leads to a repetition of the sludging phenomena in the post capillary venules of the splanchnic microcirculation. The effect of the sludging of blood in both the hepatic and portal circulations is to reduce even further that volume of blood which is effectively circulating in a situation where the total blood volume has already been seriously depleted.

Thus, a vicious cycle is set up wherein one element leads to the development of the next. With hemorrhage, decreased blood volume stimulates the production of catacholamines; the latter produce an increased rate of hepatic blood flow with an increased portal vein pressure and an increased resistance across the liver. The increased hepatic venous resistance further increases the portal vein pressure which in turn leads to hindrance to blood flow from the splanchnic area. With continued blood loss the capacity of these initial compensatory reactions are strained to a new maximum.

There follows a critical situation with respect to viscosity and the diameter of the vessels. The hepatic sinusoid now becomes the weak chain in the link, and at a critical level aggregation of red cells with congestion of the hepatic sinusoid further compromise the flow of blood through this organ, backing up of blood into the portal circulation leading to loss of blood from the effective circulating volume by aggregation of red cells in the microcirculation. This leads to an intensified stimulation for further secretion of catacholamines.

With congestion and impaired circulation through the liver there is also a functional impairment of the metabolic and biochemical capacities of the liver, and in consequence of this, acidosis, high potassium, accumulation of fixed acids and other breakdown products of metabolism appear in the circulation plasma to produce their own insidious consequences.

Paradoxically, the addition of blood to this animal after the critical stage is reached, instead of producing amelioration of the situation, may actually hinder or retard the lesion. The addition of more blood to a system which has al-

ready had a block or a dam at the level of the hepatic sinusoid produces inevitably a further sequestration or pooling of blood in the splanchnic area. Although temporary benefit is observed in the blood pressure this is relatively short lived.

The cycle of events leading to irreversible shock might be more logically attacked by attempting to reverse the microcirculatory defect. Encouraging preliminary studies using low viscosity dextran have shown reversal of the sludging or red cell aggregation and restoration of organ flow in the **in situ, in vivo** perfused liver[8]. This suggests the need for more experimental work in this area as well as a program for the clinical evaluation of this and other remedial agents.

The author is aware of many difficulties, objections and incompletely studied areas in the general scheme of the pathophysiology of shock as developed. Work is presently in process to fill some of these gaps: studies are being carried out on the effects of serotonin, neural stimulation to splanchnics and hepatic nerves, blocking agents, measurements of tissue hemoglobin, etc. The necessity of evaluating some of the critical parameters in patients who are in various stages of shock is fully appreciated. Moreover, it is anticipated that the proper elucidation of all the factors involved in the development of irreversible hemorrhagic shock is more than enough to fill a life time of investigation.

Results of these initial studies were integrated into a tentative scheme which is consistent with previously observed data. The central core of thought is that there is no single cause of the "state of shock"; rather, shock (admittedly a poor term) is a syndrome which may be induced by a multiplicity of specific causes. More important than the enumeration of these initiating causes (blood loss, bacterial endotoxins, biochemical blocking agents as fluoroactate, etc.) are the elucidation of the sequence of the events and mechanisms involved in the development of the clinical syndrome. A cycle of events are suggested as a way of interrelating these events and factors to the downward clinical course.

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CARCINOMA OF THE FEMALE BREAST

A survey of all cases treated surgically at Mount Sinai Hospital from January to June of 1960.

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This report presents a survey of carcinoma of the female breast treated surgically from January to June of 1960 at Mount Sinai Hospital. The survey is a retrospective review of all clinical charts and pathologic reports of these cases.

During this period, 27 cases of carcinoma and 80 non-malignant lesions were treated surgically by procedures ranging from simple diagnostic biopsy to radical mastectomy. The lesions encountered are presented in Table I.

The most pertinent data can readily be tabulated. The types of carcinomas, the ages of patients, location of lesions, duration of symptoms prior to therapy, size of lesion and clinical and pathologic evidence of metastases in axillary nodes are provided in Tables II, III and IV and in Figure 1.

The terminology is based on the pathologic diagnosis.

The distinction between ductal carcinoma and other carcinomas of particular types warrants emphasis. Ductal carcinoma is the "garden variety" or common type; this group is more homogeneous than other groups, and makes up roughly two-thirds of this and other series of cases. It includes lesions described as infiltrating ductal carcinoma, scirrhous carcinoma and anaplastic carcinoma. These lesions are painstakingly separted from the carcinomas of other types be-

TABLE I

Surgically Treated Female Breast Cases, Mount Sinai Hospital January - June 1960

Carcinoma		27
Ductal Carcinomas	20	
Other Types	7	
Non-Malignant Lesions		80
Benign Tumors	20	
Fibroadenoma	16	
Cystosarcoma Phyllodes	2	
Intraductal Papilloma	1	
Granular Myoblastoma	1	
Non-Neoplastic Lesions	60	
Fibrocystic Disease	54	
(with Adenosis)	(14)	
Fat Necrosis	3	
Duct Ectasia	2	
Abscess	1	
Total Cases		107

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TABLE II

General Features — Ductal Carcinomas

Case No.	Age	Breast Lesion: Location	Breast Lesion: Size in Centimeters	Duration Symptoms	Axillary Lymph Nodes: Clinical Lympha-denopathy	Axillary Lymph Nodes: Positive Nodes Pathologic Findings Total Examined
1	31	Right lateral aspect of areola	1.9	6 Mo.	No	0/18
2	50	Left upper outer	3.0	2 Wk.	No	0/18
3	79	Left upper outer	2.0	7 Mo.	No	S
4	39	Right upper outer	6.0	4 Mo.	No	3/24
5	65	Left not described	5.0	2 Yr.	Yes	S
6	75	Right lateral aspect of areola	2.5	1 Wk.	Yes	S-2/11
7	56	Right upper outer	2.0	6 Mo.	No	8/21
8	55	Left upper outer	3.0	2 Wk.	Yes	23/24
9	47	Left upper inner	3.0	1 Yr.	No	1/18
10	46	Left medial to areola to 7 o'clock	7.0	9 Mo.	No	3/20
11	66	Left upper outer	2.3	3 Da.	No	5/13
12	62	Right upper outer	3.3	7 Wk.	Yes	0/23
13	32	Right upper and lower outer	3.5	5 Mo.	No	5/42
14	68	Right—Beneath nipple	2.0	1 Yr.	Yes	0/10
15	63	Left upper outer	3.0	Undeter-mined	No	3/21
16	32	Right upper outer	Undeter-mined	1 Mo.	Yes	2/35
17	43	Left upper outer	3.0	3 Wk.	No	22/25
18	51	Left upper inner Right—Not palpable	1.0 left 0.2 right	2 Wk.*	No	1/14 2/14
19	67	Right upper outer	4.0	2 Wk.	No	S
20	47	Right upper outer	1.5	1 Wk.	No	2/36
Av.	53.7 Years		2.9 Cm.	4.8 Mo.		

*—Discovered on routine physical examination.
S—Simple mastectomy. One case had limited node resection.

cause of major differences in behavior and prognosis, not just to stress morphologic or histologic niceties. Carcinomas of these other types are a heterogeneous group which vary as widely in behavior as in morphology. Medullary carcinomas tend to be large and bulky. Lobular carcinoma-in-situ is clinically undetectable and is found incidentally in sections of breast tissue excised for fibrocystic disease. The ductal carcinoma beneath the weeping, crusted, eczematoid nipple and areola in Paget's disease cannot always be delineated by palpation.

ing conclusions, it appears to be representative of larger series. Comparison with one reference series of 766 cases (Table V) reveals a similar incidence of ductal carcinomas, a similar age incidence and a similar percentage of axillary metastases[1]. The smaller size of lesions and the shorter duration of symptoms prior to therapy of the Mount Sinai series is discussed more appropriately later in relation to symptoms and signs.

The chief complaints of patients with ductal carcinomas are recorded in Table

TABLE III

GENERAL FEATURES

Carcinomas of Particular Types

Type	Age	Duration of Symptoms	Size	Axillary Nodes
Medullary	49	3 D	1.8 cm. aside cyst	0/12
Medullary	60	2 M	6.0 cm.	0/12
Papillary	46	2 W	+3 cm.	0/22
Intraductal Colloid	61	4 W	1.5 cm.	0/19
Pagets	63	2 Y	No gross mass	0/20
Lobular CA-in-Situ	44	None due to CA.	Gross fibrocystic disease	S
Lobular CA-in-Situ	46	None due to CA.	Gross fibrocystic disease	S

The outstandingly important difference between these two broad groups is the higher incidence of lymph node metastases and the poorer prognosis of the ductal carcinomas. In this series axillary lymph node metastases were present in 13 of 20 cases (65%) of ductal carcinomas whereas no axillary metastases were found in the other carcinomas of particular types. The presence of lymph node metastases is the most important single factor of prognostic significance in breast carcinoma.

Although this small series does not per se provide a statistical basis for sweep-

VI. Only one of these cases was first detected by routine physical examination of an asymptomatic patient. Of the remaining nineteen, one patient reported with an initial complaint of weakness; all of the others presented with symptoms localized to the breast as outlined in Table VII. Approximately 15% of the patients had an initial complaint of nipple discharge and 20% had pain or tenderness of some sort. Only one case had a bloody nipple discharge.

Carcinomas of particular types are too heterogeneous a group to warrant analysis of symptomatology.

In practice the most important diagnostic feature of breast carcinoma is the **mass.** By the time of surgery all of our cases of ductal carcinoma had a palpable mass. The location of the carcinomas is depicted in Figure 1. Other symptoms are usually relevant largely in relationship to their association with the mass. Although Haagensen[1] cites instances of detection of masses as small as 0.5 cm. by patients and by examining physicians, only one of our eighteen cases of ductal carcinoma had a mass less than 1.9 cm. on pathological examination. The clinical estimate of tumor size is usually at least 1 cm. greater than the measurement reported by the pathologist.

Haagensen[1] has cited data which suggest that during the past several decades patients seek medical advice sooner and present with smaller lesions. Such a trend explains the shorter duration of symptoms and the smaller average size of lesions in the Mount Sinai series of 1960 contrasted with the Presbyterian series in Table V which includes cases from 1915 to 1942.

In order to ascertain whether obvious changes in our breast carcinoma cases have occurred in more recent decades, the age, size of lesion and the duration of symptoms of cases of ductal carcinoma during an equivalent time period in 1940, 1950 and 1960 were compared.

The only significant change detected in our available material in 1940, 1950 and 1960 (Table VII) is the shortening of the duration of symptoms prior to treatment from slightly over one year in 1940 to less than 6 months in 1950 and 1960.

TABLE IV

The Axillary Nodes in Carcinoma of No Special Type

THE CLINICAL ASPECT		
Clinically palpable nodes*		6
Metastases confirmed pathologically	3	
No metastases pathologically	2	
Simple mastectomy. No nodes submitted	1	
Clinically non-palpabale nodes		14
Metastases found pathologically	10	
No metastases pathologically	2	
Simple mastectomy. No nodes submitted	2	
		20
THE PATHOLOGIC ASPECT		
Pathologically demonstrable metastases		13
No clinically palpable nodes	10	
Clinically palpable nodes	3	
Pathologically negative for metastases		4
No clinically palpable nodes	2	
Clinically palpable nodes	2	
No nodes submitted. Simple mastectomy		3
		20

* One simple mastectomy with nodes removed.

Indeed 8 of 20 patients in 1960 had symptoms less than one month prior to surgical therapy. Nevertheless, the shorter average duration of symptoms is not associated with a reduction of average size of the lesion as measured by the pathologist during the immediately past twenty years.

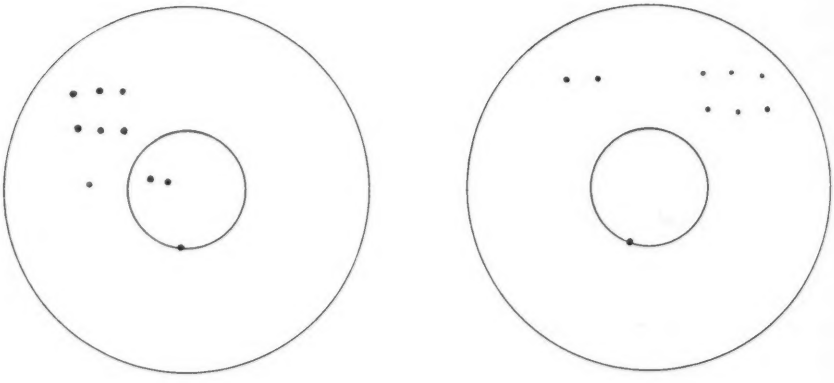
In our opinion, this study further demonstrates the importance of recognition of symptoms and signs by the patient. It is almost always the patient who first recognizes the symptoms or signs which lead to diagnosis and therapy. Patient awareness and co-operation depend upon patient education. Thus there is an obvious continuing need to educate women about the symptoms and signs of breast carcinoma with emphasis on self examination. The most valuable source of cancer education programs is the American Cancer Society and its affiliates. In our opinion, the great value of such programs can be measured by the shortening of the duration of symptoms of patients prior to seeking medical advice.

In no instance in this series was a fallacious policy of watchful waiting adopted by an attending physician. No example of delay in therapy attributable to a physician was encountered.

No correlation between the size of the primary tumor and the number of node metastases was apparent in this series. In studies of very much larger series from the literature there is some correla-

Fig. 1

Location of 21 Ductal Carcinomas in 20 Patients



RECAPITULATION

Upper outer	12
Periareolar	4
Upper inner	2
Upper and lower outer	1
Not specified	2

tion between primary size and node metastases[2]. When small primary tumors are associated with prominent node metastases, the tumor is presumed to be more highly malignant.

Clinically detectable lymphadenopathy correlates poorly with pathogically demonstrable metastases in axillary nodes in this series. In 10 cases metastases were found by the pathologist although no clinically detectable lymphadenopathy was present. In 2 cases clinically palpable lymph nodes contained no metastases. In one 65 year old female clinically positive lymphadenopathy could not be evaluated pathologically because only a simple mastectomy specimen without nodes was available. In 4 cases without clinical lymphadenopathy, no metastases were found pathologically in 2. The other 2 were treated by simple mastectomy and no nodes were available for pathologic study. In 3 cases of clinically detectable lymphadenopathy, metastases were confirmed pathologically.

In this series as in most larger series only pathological examination of nodes provides accurate data regarding metastases. The reason for most of these discrepancies is the fact that clinical palpability depends on the size and consistency of a node within a bed of adipose tissue. Reactive hyperplasia of nodes may produce prominent nodes in the axilla of thin patients. Small metastases may be impossible to feel even in thin patients. Deeply placed nodes moderately enlarged by metastases may not be detected on palpation by an experienced observer in an obese patient.

Any attempt to evaluate the accuracy of clinical diagnosis must give due consideration to several important factors.

First, the eighty non-malignant lesions admitted for surgical excision or biopsy are not representative of the incidence of these lesions in general practice. More overtly benign lesions, particularly fibrocycstic disease, are excluded by family doctors and consulting surgeons. The benign lesions which pass preliminary screening for surgery include a high incidence of cases of fibrocystic disease with at least one firm, prominent mass (the syndrome of the predominant lump) which the surgeon cannot distinguish with reasonable certainty from carcinoma. This is reflected by the fact that almost half of the cases reported as fibrocystic disease by the pathologist were described preoperatively by the surgeon with the non-committal term "tumor."

Second, carcinoma may be masked by coexistent benign disease. At least five carcinomas in this series were completely masked by coexistent fibrocystic disease and hence first diagnosed by the pathologist. In the large series of 1434 local excisions for clinically benign lesions at Memorial Hospital between 1945 and 1948, frozen section examina-

TABLE V

Comparison of Mount Sinai Hospital Cases of Ductal Carcinoma with a Large Reference Series

Hospital	Period of Study	Number of Cases of Ductal Carcinomas of Breast Cancer	Percentage of Total Cases	Average Age	Average Size	Duration Symptoms in Months	Percentage of Axillary Metastases
Presbyterian New York City	1915-1942	766	70.7	50	4.9	8.3	66.4
Mount Sinai Chicago	1960	20	74.0	53.7	2.9	4.8	65.0

tion revealed 154 cancers (approximately 10% of the cases). Of particular importance is the fact that cases which were clinically undiagnosed had a better salvage rate than the group of clinically overt carcinoma.

Third, it is generally acknowledged that certain lesions sometimes cannot be satisfactorily differentiated from carcinoma without biopsy and frozen section pathological examination confirmed by routine sections. In this study such lesions included fibrocystic disease with the syndrome of the predominant lump, adenosis of the sclerosing variety, granular myoblastoma, fat necrosis and cystosarcoma phyllodes.

Routine diagnostic biopsy at this institution is usually a total excisional biopsy whenever feasible. This ideal can almost invariably be achieved when the surgeon deals with firm, discrete lesions of moderate size. Several surgeons, however, prefer incisional biopsy.

A brief review of frozen section diagnosis is necessary at this point. Frozen section diagnosis has been highly accurate in this series. No false positive diagnoses were made. All submitted cases of carcinoma of no special type were correctly diagnosed as were all other submitted carcinomas. Lobular carcinoma-in-situ was suspected but paraffin section confirmation was requested. To offer maximum assistance to the surgeon it is the policy of the Department of Pathology to offer unequivocal positive or negative diagnoses or to withhold interpretation pending rapid paraffin sections if diagnosis is equivocal. The Department is particularly wary in cases of sclerosing adenosis, papillary intraductal lesions and lobular carcinoma-in-situ. Because of the crucial importance of frozen section diagnosis, this task is done only by senior pathologists.

It must also be noted that early during the study period several lesions which were not submitted for frozen section were first diagnosed as carcinoma by the pathologist.

Needle aspiration biopsy and cytologic examination of nipple discharge were not done in any case during the study period. We have regarded them as being of insufficient diagnostic value to warrant further evaluation by us.

Although it would seem a simple matter to determine the accuracy of clinical diagnosis by comparing the admission diagnosis of the referring physician and the pre-operative diagnosis of the operating surgeon with the final pathologic diagnosis, in practice it proved impossible to do so for three reasons which have already been discussed. Non-committal terminology was commonly used prior to actual biopsy. The unqualified term "tumor" was utilized in the records to cover indeterminate or suspicious masses warranting biopsy; it was the pre-operative diagnosis in approximately half of the carcinoma cases and half of the cases of fibrocystic disease. What may superficially appear as a diagnostic dilemma is resolved on the clinical level very simply. In practice, every indeterminate breast mass warrants biopsy by the surgeon and frozen section examination by the pathologist.

All carcinomata except lobular carcinoma-in-situ were treated preferentially by radical mastectomy. In four instances ductal carcinoma was treated by simple mastectomy usually because of senility, gravity of surgical risk or presence of distant metastases. No cases have been treated by supra-radical mastectomy.

Post-operative rehabilitational exercise

TABLE VI

Chief Complaint in Ductal Carcinoma

Mass only*	11
Nipple discharge	
Bloody	1
Not bloody	2
Pain and/or tenderness	4
Weakness	1
No complaint**	1

* In all cases a palpable mass was detected preoperatively by the surgeon.

**Discovered on routine examination.

is of great importance after radical mastectomy. Many of the cases were referred to the Department of Physiotherapy for supervised exercise.

The staff is well aware of the psychosomatic importance of mastectomy. The responsibility of the physician in this sphere is not dismissed merely by selection of a new breast form. The art of managing the psychologic aspects of the problem is beyond the scope of this discussion.

Irradiation in this series was exclusively a post-operative measure. It is requested routinely by the surgical department for all cases with axillary lymph node metastases. There are, however, other important indications. These include invasion of adjacent tissues, such as the skin, the pectoralis fascia or muscles, and location of the primary tumor in one of the inner quadrants or the central position. Post-operative irradiation should also be carried out in cases showing intra-vascular extension, be it lymphatic or venous, as well as for the highly anaplastic types of carcinoma.

The Department of Therapeutic Radiology usually accepts all referred post-operative cases for treatment unless particular contra-indications are present. Radiotherapy is varied in accord with the indications. In most of these cases, irradiation is directed towards both the regional lymph node areas and the chest wall, delivering tumor doses of 3,500r to the axilla, 3,000r to the supraclavicular area in 30-35 days, and a maximum of about 2,500r to the chest wall in 17-21 days. In cases with histologically negative nodes or equivalent findings, the lymph nodes but not the chest wall, are irradiated. Special attention is paid to the internal mammary chain of nodes in tumors located in the medial half of the breast.

Pre-operative irradiation was not utilized for any case in this series. It is rarely planned as such, but usually evolves as the primary treatment in seemingly inoperable cases which may thereafter be re-evaluated as suitable for surgery.

Castration is rarely done as an immediate post-mastectomy prophylactic measure. Three patients in this series were subjected to prompt post-mastectomy oophorectomy. In 3 additional cases, castration was carried out by pelvic irradiation. No dependable objective criteria for selection of patients for castration are available although a combination of youth and extensive axillary lymph node metastases or anaplastic type of growth are among the indications suggested. This reflects the broader biologic fact that hormonal dependance cannot be determined in individual cases at present by morphologic criteria.

Routine prophylactic castration in all pre-menopausal cases was recommended at the recent Fourth International Cancer Conference in Minneapolis but current practice in this institution favors the alternative of reserving castration until the appearance of systemic metastases or local recurrence.

TABLE VII

Comparison of Features of Ductal Carcinomas at Different Time Periods

Year	Cases	Age	Size in Cm.	Duration in Mo.
1940	17	47	3.1	12.4
1950	15	54	3.3	2.8
1960	20	53.7	2.9	4.8

SUMMARY

The panorama of symptomatology, diagnosis and therapy of all cases of breast cancer treated at Mount Sinai Hospital from January to June of 1960 is reviewed in an attempt to summarize concepts of practical importance to physicians dealing with breast cancer which is the commonest malignant tumor of women.

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PHAGOCYTIC DEFENSE MECHANISM

NORMAN A. LASKY, A.B.*

Phagocytosis may be broadly considered as the process of ingestion and digestion by cells. Most of the cells of the body which are concerned in some way with nutrition possess this property and the particles which are ingested in this process include other cells, bacteria, bits of dead tissue, and many foreign particles.

In the 1850's this phenomenon was first observed by zoologists, but it was not until 1886 that this process was aptly named and described by Elias Metchnikoff. Metchnikoff was a Russian scientist who, while observing intracellular digestion in a starfish, noticed the presence of many "wandering cells" which exhibited amoeboid motility. The function of these cells at first appeared vague, but Metchnikoff postulated that they must be connected in some way with the defense mechanism of the body.

This theory was supported by the accumulation of these "wandering cells" around a rose thorn, which he introduced into the starfish skin. After other similar experiments, he concluded that these cells would appear in any area of local injury or infection for defense against harmful microorganisms.

Metchnikoff named these cells phagocytes, from the Greek **phagein**—to eat, and **kytos**—hollow cell. (DeKruif.) The phagocytes, along with cells producing antibodies, sinusoids, lymphoid tissue, lymphoid organs, and lymphoid vessels, comprise the "defense system of the body." (Bargmann.)

The phagocytic defense mechanism may be seen to consist of three phases: (1) chemotaxis, (2) phagocytosis per se, or engulfment, and (3) digestion of ingested materials. This paper is devoted mainly to the second phase, phagocytosis per se, and also to a slight degree with the preparatory phase of chemotaxis.

Chemotaxis is a reaction by which the direction of locomotion of cells or organisms is determined by substances in their environment. Chemotaxis is positive if the direction is toward the stimulating substances and negative if it is away from the stimulating substance. Therefore, this reaction may be considered a directional response to chemical stimuli which plays a major role in determining the rates at which particles suspended in circulating blood are brought in contact with phagocytes. Consequently, there must also be an important relation to the selectivity of phagocytosis. This will be dealt with more completely under opsonization and the effect of the particle in the environment on phagocytosis. (McCutcheon.)

What then is the relation between chemotaxis and phagocytosis per se? First, it must be stated that chemotaxis does not necessarily precede phagocytosis. That is, it is not true that a bacterium must attract a phagocyte from afar before it can be engulfed. Many particles, such as inorganic carbon and silica, do not attract the phagocytes, but rather are engulfed after chance encounters with them. Also, monocytes, the most voracious of the phagocytes, are not known to be attracted by substances in their environment. It should also be mentioned that chemotaxis is not always followed by phagocytosis. Polymorphonuclear leukocytes are attracted by dead

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cells; however, they do not engulf them after this positive chemotaxis occurs.

The mechanisms of chemotaxis and phagocytosis are also different. Phagocytosis depends on the ability of the leukocyte to spread on the object to be engulfed. This spreading is greatly aided by specific antibodies known as tropins. Tropins, however, play no part in chemotaxis, and it is more than likely a nonspecific reaction which is stimulated by antigens but not by antibodies. Chemically, chemotaxis is not a spreading reaction like phagocytosis, but it is dependent upon complicated sol-gel transformations in different parts of the cell body. (McCutcheon.)

The second phase of the phagocytic defense mechanism, phagocytosis per se, should be viewed from three aspects: (1) the phagocyte, (2) the ingested particle, and (3) the environment in which ingestion takes place.

Phagocytes may be divided into two classes: (1) the microphages and (2) the macrophages. The former class consists of the polymorphonuclear leukocytes, which are small and ingest mainly bacteria. The neutrophilic polymorphonuclear leukocytes are more phagocytic than the eosinophils or basophils; in fact, the eosinophils are rarely found to be phagocytic. The younger of the mature granulocytes are the most effective phagocytes, and an increase has been noticed in the phagocytic potential of these granulocytes in anemic animals.

Diminished phagocytic activity has been observed in granulocytes among infants, myeloid leukemia patients, animals made deficient in vitamins B or C or in proteins, patients on corticotropin or cortisone therapy, and in stored blood. Phagocytic activity also has been noticed to be dormant under 15°C, increased as 37°C is approached and decreased above 40-42°C. (Wright and Dodd.)

The second class of phagocytes, the macrophages, are large mononucleated cells which are largely scavengers ingesting dead tissue and degenerated cells. It is believed that monocytes are potentially macrophages when they leave the blood stream, and under pathological conditions they may fuse to form a foreign body giant cell.

However, macrophages of tissue and those developed from blood cells are also important in the disposal of noxious agents. They are scattered in the various connective tissues of the body and they make up the system of macrophages or the reticulo-endothelial system. Included in this system are: (1) macrophages of loose C. T., (2) phagocytic reticular cells of lymphatic tissue, myeloid tissue and spleen, (3) Kupffer cells in the sinusoids of the liver, (4) cell lining of the adrenal cortex and anterior lobe of the hypophysis, (5) certain perivascular cells, and (6) "dust" cells of the lung.

The functions of macrophages and microphages overlap and are co-ordinated in such a way that they even may compensate for each other. Their mechanisms are fundamentally similar and their differences may be said to be topographical rather than physiological.

Also of great importance in phagocytosis per se is the particle to be ingested. The production of antiphagocytic factors by bacteria may inhibit their ingestion. This capacity is frequently associated with bacterial virulence, or the ability to parasitize and invade animal tissues. This, therefore, may be considered a negative chemotaxis, and it is in this element of phagocytosis that the specificity of this process is determined.

There are certain substances occurring in the blood serum known as opsonins, whose action is to make microorganisms and other cells more attractive to the phagocytes. This has been known since Metchnikoff's time as the opsonic function of antibody. Certain opsonins are present in normal serum and act on all organisms. Others are formed in response to certain stimuli and are specific for one bacterial species only. There are also some non-antibody proteins which are not found in the serum, such as fibrin and globin, which act as nonspecific opsonic agents.

Other substances such as tannin, certain enzymes, and certain viruses which increase erythrocyte phagocytosis in normal serum are not considered opsonins, yet they produce the same effect. These substances, when introduced onto the

erythrocyte's surface, bring certain antigens to the cell surface, which in turn attracts normal antibodies in normal serum. These antibodies then proceed to act as opsonins. (Wright and Dodd.)

Knisely, in his study of selective phagocytosis in the liver sinusoids of frogs, observed that some kinds of particles were not ingested by the hepatic phagocytes (the Kupffer cells). To determine the cause of this selectivity he injected Higgins India Ink into the blood of the frogs. The ink particles immediately received a coating which separated them from the blood stream. The outer surface of this coating was not sticky to red cells or normal undamaged vascular endothelium. The hepatic phagocytes selectively ingested those particles which were coated, regardless of what was in them. Knisely, therefore concluded that the presence of the coating was sufficient to make the particles ingestible, and this coating determined the selectivity of ingestion by these stationary phagocytes.

Another important element in the consideration of this phase of the phagocytic defense mechanism is the environment. Most standardized tests in which phagocytosis is viewed on a smooth glass surface do not offer the proper **in vitro** environment for correct observation of this process. For this is not the same as the rough surfaces present **in vivo.** This factor is mainly responsible for the erroneous conclusions often derived from observations of phagocytosis. (Wright and Dodd.)

In the body, the microphages move intravascularly, in the blood current, where they are responsible for stopping the bacteria causing acute infections. On the other hand, bacteria causing chronic infections do most of their damage intracellularly. The microphages may be seen moving in the blood current in a rolling fashion and at this point are not actively phagocytic. However, the entrance of bacteria into the blood causes these granulocytes to stick to the endothelium of capillaries and assume amoeboid motility. They are then potentially phagocytic.

Intravascular phagocytosis may be accomplished in three ways without specific opsonization: (1) the particle may be trapped by a phagocyte against the endothelium, (2) the particle may be caught between two adjacent leukocytes, (3) or the particles may be caught in small fibrin deposits along the capillary wall and then phagocytized by the granulocytes. Therefore, intravascular phagocytosis varies inversely with the rate of blood flow; the slower the blood flow, the greater the chance of particles encountering phagocytes and being trapped by them.

Surface phagocytosis depends on the existence of rough, sticky surfaces so the leukocyte can successfully pin the particles and engulf them. Consequently, the compact tissue of the spleen, lymph nodes and liver, is most suitable. and open cavities, where chance of contact is not as great, are less effective. (Wright and Dodd.)

Knisely has observed that in the liver of a frog, all sinusoid lining cells are phagocytic and when bumped or rubbed by a coated particle contain that particle instantly. The amoeboid engulfment usually attributed to the phagocytes was not exhibited. Also, in the case of these coated particles, the rate of the blood flow did not have to be slow or stationary for phagocytosis to take place.

The mechanisms of phagocytosis deserves brief mention at this point. They are classically attributed to surface energy and electrostatic charge.

In experiments dealing with the surface energy, the introduction of surface activating agents, substances which lower surface tension, onto the phagocyte or the particle, produced different results. When the surface of the phagocyte was treated there was an increase in phagocytic activity, whereas, treatment of the particle with the same agents resulted in a decreased activity. Treatment of both the phagocyte and the particle resulted in no change in phagocytic rates. However, the introduction of non-surface activating agents of similar chemical composition produced the same results. It was, therefore, concluded that there is little relation between lowering of surface tension and phagocytic stimulation. It should be mention-

ed, however, that there are no known methods for determining interfacial tension between the surfaces of the phagocyte and the particle.

Studies concerning the electrostatic charges on the particle and the phagocyte have resulted in similar inconclusive observations. Serum is known to decrease the electrostatic charge on bacteria, and these cellular particles with lessened charge are more susceptible to phagocytosis. However, it was also observed that leukocytes are negatively charged. Therefore, the validity of this theory has been questioned because these two negatively charged particles are attracted to each other. But if means were available for measuring localization of charges rather than total surface charges, these theoretical mechanisms might be more tenable. In fact, there is some evidence that the charges on the phagocyte's surface do vary in a mosaic pattern. It has also been suggested that this mechanism is not physical in nature, but rather biochemical. (Wright and Dodd.)

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« ABSTRACTS »

POZZA, G., GALANSINO, G., HOFFELD, H., and FOA, P. P. *(Dept. of Physiol. and Pharmacology, The Chicago Medical School):* Stimulation of Insulin Output by Monosaccharides and Monosaccharide Derivatives. Am. J. Physiol. 192(3): 497-500, 1958.

The ability of various sugars and closely related substances to stimulate insulin secretion was studied by means of pancreatic-femoral cross-circulation experiments between hepatectomized donor dogs and normal recipients. In other experiments, the test substance was injected directly into the pancreatic artery of normal dogs. The administration of *d*-galactose or *d*-ribos was followed by a prompt hypoglycemia, suggesting insulin secretion; *d*-arabinose caused an unexplained delayed hypoglycemia, while *d*-fructose, *d*-mannose, *d*-xylose, *l*-arabinose, 3-methylglucose, *d*-glucosamine, galacturonic acid and saline had no effect. The tentative hypothesis that insulin secretion is stimulated by sugars which are both utilizable and insulin-sensitive is offered. No relationship between chemical structure and ability to cause insulin release was found.

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RYAN, A. H., SOREN, S., COHEN, S. G. and WEIL, W. *(Dept. of Physiol. and Pharmacol.):* Effectiveness of a Chelating Agent in Terminating Cardiac Arrhythmias Induced by Acetyl Strophanthidin.

Arrhythmias induced in cats by divided doses of acetyl strophanthidin (AcS) usually terminate automatically within a few minutes. Their duration may be greatly prolonged by 2 procedures: first, by causing repeated episodes of arrhythmias, in which case, the duration usually becomes much longer with smaller doses of drug and, second, by giving digitoxin (0.175-0.2 mg/kg i.m.) on the day prior to the injections of AcS, the latter method resembling the conditions under which AcS is employed clinically as a digitalis tolerance test. Under the former procedure ethylene diamine tetra-acetic acid di-potassium salt (K-EDTA) in doses of 5 to 20 mg/kg is usually effective in terminating the arrhythmias (13 successes and 4 failures with 11 cats). When a near toxic dose of digitoxin preceded the toxic dose of AcS, the latter drug was eliminated independently at approximately the same percent of drug per unit of time as when present alone, but the arrhythmias persisted much longer. In such experiments the effect of K-EDTA was less satisfactory (7 successes and 12 failures). The mean lethal dose of AcS, when K-EDTA was given, concurrently, intravenously, was .1717 ± S.E. 0.0092 mg/kg for 10 cats, while the mean lethal dose of AcS alone, in 5 control cats, was 0.1337 ± S.E. 0.0068 mg/kg. The difference between the two means is statistically significant.

DASLER, WALDEMAR and MILLISER, RUSSELL V. *(The Chicago Medical School):* Osteolathyrogenic-like Action of Some Chemicals.

Feeding sweet peas, B-aminopropionitrile (BAPN), or aminoacetonitrile to weaning rats produces osteolathyrism. Two non-nitriles have previously been reported to produce similar skeletel changes, viz., semicarbazide (*Proc. Soc. Exper. Biol. & Med.*, in press) and B-mercaptoethylamine (cysteamine) (*ibid.* 88:196, 1955). The lathyrogenic activity of the latter compound has been questioned (Ponseti *et al., ibid.* 92:366, 1956), but was confirmed again with several different commercial lots of the hydrochloride, the purity of which was checked by iodimetric titration. Cystamine dihydrochloride, when fed at 0.3-0.5% levels, and acetone semicarbazone produced similar skeletal lesions. Preliminary microscopic observations on aortas and bones will be reported. Less severe spinal curvatures were produced in rats by p-hydrazinobenzoic acid, 4,4-diphenyl semicarbazide, 1,5-diphenyl carbazide, and 1,3-diethyl-2-thiourea. These substances caused little or no exostosis formation on mandibles or femurs. Thiosemicarbazide produced a severe kyphosis different in character from the spinal curvatures produced by any of the above substances. Rats fed thiosemicarbazide developed a severe dorsal hump in the lower thoracic area without the sharp ventral dips seen in rats receiving BAPN or semicarbazide. Roentgenologically the vertebrae appeared normal and no femoral or mandibular exostoses were observed. These animals also exhibited severe atrophy of the muscles. (Aided by PHS Grant No. A-1427.)

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POZZA, GUIDO, GALANSINO, GIORGIO, HOFFELD, HARVEY and FOA, PIERO P. *(Dept. of Physiol. and Pharmacol.,):* Stimulation of Insulin Output by Monosaccharides and Monosaccharide Derivatives. Am. J. Physiol. 192(3):497-500. 1958.

The ability of various sugars and closely related substances to stimulate insulin secretion was studied by means of pancreatic-femoral cross-circulation experiments between hepatectomized donor dogs and normal recipients. In other experiments, the test substance was injected directly into the pancreatic artery of normal dogs. The administration of *d*-glucose, *d*-galactose or *d*-ribose was followed by a prompt hypoglycemia, suggesting insulin secretion; *d*-arabinose caused an unexplained delayed hypoglycemia, while *d*-fructose, *d*-mannose, *d*-xylose, *l*-arabinose, 3-methylglucose, *d*-glucosamine, galacturonic acid and saline had no effect. The tentative hypothesis that insulin secretion is stimulated by sugars which are both utilizable and insulin-sensitive is offered. No relationship between chemical structure and ability to cause insulin release was found.

DASLER, WALDEMAR and MILLISER, RUSSELL V. *(Depts. of Biochem. and Pathol.):* Experimental Lathyrism in Mice Fed Diets Containing Sweet Peas or B-aminopropionitrile.

1) Weanling, male, albino mice were more resistant to the skeletal effects of diets containing sweet peas or B-aminopropionitrile (BAPN) than were rats. They also showed a much greater individual variation in susceptibility to the skeletal effects of such diets. At sufficiently high levels of BAPN, however, both skeletal and aortic lesions occurred. The mocroscopic changes occurring in the aorta are described in detail. 2) Paraphymosis also occurred, but the paralysis and urinary retention often seen in rats receiving similar diets were not observed.

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DASLER, WALDEMAR *(Dept. of Biochem.):* Production of Semicarbazide of Gross Skeletal Changes in Rats Similar to Osteolathryism.

Semicarbazide HCl, when fed to weanling male rats, produced gross skeletal lesions similar to those of osteolathyrism. Aortic damage seemed to develop more slowly and to be less severe than in rats treated with BAPN or aminoacetonitrile and having skeletal lesions of comparable severity.

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DASLER, WALDEMAR and MILLISER, RUSSELL V. *(Depts. of Biochemistry and Pathology):* Osteolathyrogenic Action of Mercaptoethylamine and of Cystamine.

The osteolathyrogenic action of β-mercaptoethylamine (cysteamine), when fed to rats as the hydrochloride, was confirmed. Cystamine was found to have similar properties. Gross and microscopic skeletal changes were similar to those produced by osteolathryrogenic nitriles. Aortic lesions were less severe than when β-aminopropionitrile is fed, but medial swelling and lysis of elastic fibers were observed. These substances showed a narrow range of osteolathyrogenic activity, a dietary level of 0.2% cystamine 2HCL causing only very slight spinal curvatures, while a level of 0.5% was rapidly lethal to most animals.